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European Collaborative Research on Mosaicism in CVS (EUCROMIC)—Fetal and Extrafetal Cell Lineages in 192 Gestations With CVS Mosaicism Involving Single Autosomal Trisomy

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Cytogenetic information on cells from cytotrophoblast, villus mesenchyme, and one or more fetal tissues was available for 192 gestations with mosaicism or non-mosaic fetoplacental discrepancy involving a single autosomal trisomy in the chorionic villus sample (CVS), registered in a collaborative study (EUCROMIC) during the period 1986–1994. In order to identify predictors of confined placental mosaicism (CPM), generalized mosaicism and/or uniparental disomy (UPD), distribution of the mosaic and non-mosaic aneuploid cell lines in the different fetal and extrafetal cell lineages were analyzed. Data were related to existing hypotheses on mechanisms leading to fetoplacental discrepancies and early extraembryonic cell differentiation. Trisomy 21 mosaicism was the one most frequently confirmed in the fetus. Non-mosaic trisomy 13, 18, and 21 in the villus mesenchyme indicated the presence of a trisomic cell line in the fetus proper. Non-mosaic trisomy 2, 7, and 16 in villus mesenchyme was always found with concomitant mosaic or non-mosaic trisomy in the cytotrophoblast, but was never recovered in the fetus. Mosaic trisomy 3, 7, and 20 was predominantly restricted to the cytotrophoblast, mosaic trisomy 2 to the villus mesenchyme. Trisomies 15 and 16 were most often found in both cytotrophoblast and villus mesenchyme and not in fetal cells. This supports the hypothesis that mosaicism/discrepancy for trisomies 15 and 16 results more often than for the other trisomies from trisomic zygote rescue, enhancing their risk for UPD. We recommend, due to the risk of fetal trisomy, amniocentesis in all gestations involving mosaic autosomal trisomy in villus mesenchyme. In gestations with mosaic or non-mosaic autosomal trisomy in both cytotrophoblast and villus mesenchyme we recommend, in order to exclude fetal trisomy and/or UPD, depending on the chromosome involved, further examination by amniocentesis, ultrasound and/or test for UPD. We also recommend, due to a small but not negligible risk of false negative and false positive diagnoses, not to solely use direct preparation. Am. J. Med. Genet. 70:179–187, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: **chorionic villus sampling; mosaicism; cell lineage; autosomal trisomy; uniparental disomy; prenatal diagnosis**

INTRODUCTION

In 1–2% of chorionic villus samples (CVS), karyotyped for prenatal diagnosis, a mosaic or non-mosaic chromosome complement differing from that of the fetus is observed [Vejerslev and Mikkelsen, 1989; MRC Working Party, 1991; Ledbetter et al., 1992; Teshima et al., 1992; Pittalis et al., 1994; ACC Working Party on Chorionic Villi in Prenatal Diagnosis, 1994]. Mosaicism with a diploid and a trisomic cell line is thought to originate either through postzygotic, mitotic nondisjunction or anaphase lag in conceptuses originally diploid or through mitotic loss of a supernumerary chromosome in subsequent divisions in trisomic conceptions (trisomic zygote rescue). However, the extent and distribution of the aberrant cell line in the conceptus is thought to depend on the timing of the chromo-

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Centers participating in EUCROMIC are listed in the Appendix.

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somal mutation, the cell lineage affected, the chromosome(s) involved and the viability of the mutation [Kalousek, 1985]. Differentiation of specific types of cells and tissues in the conceptus begins at the early postfertilizational stages. It is likely that the fetus proper is derived from just three progenitor cells in the 64-celled blastocyst, the other cells giving rise to extraembryonic structures [Markert and Petters, 1978]. Therefore, theoretically, mitotic errors in early development are more likely to occur in an extrafetal than in a fetal lineage. At the 8-cell stage of embryonic development, a polarization between the centrally and the superficially localized blastomeres exists (compaction) and at about 4 days postfertilization the morula is differentiated into an inner cell mass (embryoblast, giving rise to the embryo proper and to extraembryonic structures) and an outer cell mass (trophoblast giving rise to cytotrophoblast and syncytiotrophoblast). Cell lineage-specific non-disjunction, consistent uneven compartmentalization of aneuploid cells during blastocyst development, or selection for or against particular trisomic cells in certain lineages have been suggested as possible explanations for observed non-random variation in the distribution of aneuploid cells and differential involvement of different cell types, found in conceptuses with CVS mosaicism or non-mosaic fetoplacental discrepancy [Wolstenholme, 1996]. These cell types include cells from cytotrophoblast as analyzed after direct preparation or short term incubation of the CVS, cells from the mesenchymal villus core as analyzed after long-term CVS culture, cells from fetal skin, respiratory and genitourinary tracts and amniotic membrane as obtained on cultivation of amniocentesis samples, fetal blood cells as obtained on cordocentesis or postnatally and fibroblasts from aborted fetuses.

If the CVS mosaicism or non-mosaic fetoplacental discrepancy involving a trisomy has originated through non-disjunction with subsequent reduction to disomy, there is a theoretical risk of 1/3 for uniparental disomy (UPD) in the fetus. The present paper is an analysis of distribution of mosaic and non-mosaic cell lines for specific single autosomal trisomies in the different fetal and extrafetal cell lineages in gestations with CVS mosaicism or non-mosaic discrepancy. Data from 1986 to 1994 were collected in the European concerted action, EUCROMIC. Our aim was to determine whether it is possible, from the prenatal cytogenetic findings on the CVS to identify those gestations with CVS mosaicism or fetoplacental discrepancy which are at increased risk of true fetal mosaicism or, theoretically, UPD. This would be helpful in counseling after prenatal diagnosis.

MATERIAL AND METHODS

Data on CVS mosaicism and fetoplacental discrepancies collected for the period 1986–1994 by 79 European laboratories (Appendix) were compiled. The compiled data consists of all data from the period, registered at EUCROMIC before February 1, 1996; i.e., both cases never included previously in publications and cases previously published by single or multiple European centers as case reports, case series, or national studies from part of the period. Data on CVS mosaicism from 1986–1987 were previously published [Vejerslev and Mikkelsen, 1989], but are also included in the present paper. Two questionnaires were completed yearly by each center: a summary questionnaire and a questionnaire on cytogenetic details and pregnancy outcome of each gestation with CVS mosaicism or non-mosaic discrepancy. The summary questionnaire contained information on the numbers of CVS received and successfully karyotyped, and numbers of direct CVS preparations/short-term incubations and long-term cultures. Chromosomal aberrations, maternal cell contamination, numbers of mosaic CVS and non-mosaic discrepancies were recorded. Mosaicism was defined as 1) a normal cell line and two or more cells with identical chromosomal aberration in direct preparation/short-term incubation and/or long-term culture, or 2) a normal cell line in direct preparation/short-term incubation and a chromosomal aberration in at least two mitoses from long-term culture, or vice versa. Samples where the trisomic cell line was not found in more than one culture vessel (pseudomosaicism) were not included in the study. Non-mosaic fetoplacental discrepancy was defined as a normal cell line or a cell line with a numerical or structural aberration by CVS and a different karyotype in cells from amniotic fluid, fetus, or newborn. The case questionnaire requested information on the chromosomal constitutions encountered by direct CVS preparation/short-term incubation, by long-term culture, by amniocentesis, in fetal tissue, placental tissue after abortion/birth, blood or skin from the neonate/fetus, and on the outcome of the pregnancy. Data were entered in and extracted from the computer program Paradox Win 1.0. The database was validated monthly by standard queries and after all data entries.

All cases involving single autosomal trisomies with information on the karyotypes of both direct CVS preparation and long-term culture and a minimum of one karyotype of fetal cells (including amniocytes) were grouped according to the cell lineages affected and the frequency of each group was calculated. The number of gestations with mosaicism or discrepancy involving single autosomal trisomies was calculated for each group by the autosome involved, including the frequency of cases occurring in a mosaic vs. a non-mosaic form. Probabilities of mosaic or non-mosaic trisomy in the fetus were calculated for each combination of placental cell lineages affected.

RESULTS

A total of 92,246 CVS were successfully karyotyped by the 79 laboratories reporting during the 1986–1994 period. CVS mosaicism or non-mosaic fetoplacental discrepancy was found in 1,415 (1.5%) of the samples. The aberrant cell line was a single autosomal trisomy in 650 (45.9%) of the mosaic or discrepant CVS, out of which 299 were karyotyped both after direct preparation/short-term incubation and after long-term culture. One hundred ninety-two of these had been further investigated by chromosome analysis of one or more types of fetal cells (fibroblasts from the aborted fetus and/or fetal blood from cordocentesis and/or blood or skin from the neonate and/or cells from amniotic fluid).

Therefore, the karyotype of a minimum of three different cell types was known for these 192 gestations.

The 192 gestations were divided into six groups depending on the cell type(s) in which the chromosomal aberration was found (Table I). The trisomic cell line was restricted to the placenta in 84.3% (confined placental mosaicism, CPM). In about half of the CPMs it was restricted to the cytotrophoblast, in 30% to the villus mesenchyme, and in 20% it was present in both cell lineages. There were no gestations with trisomy in the fetus proper, if the trisomic cell line was only found on direct preparation and not on culture. The probability that a chromosome abnormality found on CVS was confirmed in the fetus proper depended on the type of placental cells in which it had been found, and whether it was found in a mosaic or a non-mosaic form (Table II).

Subgrouping the groups in Table I into the specific autosomal trisomies renders statistical comparison unreliable. However, the specific autosomal trisomies did not appear to be evenly distributed among the groups (Table III). Mosaicism or discrepancy was confirmed as fetal for trisomies 8, 9, 12, 15, 18, and 20 as mosaic trisomy in the fetus, for trisomy 13 as non-mosaic trisomy in the fetus, and for trisomy 21 both as mosaic and non-mosaic trisomy in the fetus. Trisomy 21 mosaicism was the one most likely to be a true fetal mosaicism. Mosaicism or discrepancy involving trisomies 2, 3, 5, 7, 10, 11, 14, 16, 17, and 22 was never confirmed in the fetus/neonate in this series. Trisomy 2 was nearly always restricted to the villus mesenchyme and only once to the cytotrophoblast, while the opposite situation occurred for trisomy 3. Both occurred mainly as mosaic trisomies. CPM for trisomies 7, 13, and 20 were most frequently encountered only in the cytotrophoblast, while trisomy 18 CPM was also frequent in villus mesenchyme. CPM for trisomies 15 and 16 occurred frequently in both cytotrophoblast and villus mesenchyme. Non-mosaic trisomy was more frequent for CPM for trisomies 15, 16, and 20 than for the others, and was rare in CPM, restricted to the villus mesenchyme.

Discordance between the chromosomal complements of different cell types considered to be fetal, e.g., cells from amniotic fluid, blood from cordocentesis, tissue from aborted fetus, blood and/or skin from the newborn was found in three gestations. Two were mosaic trisomies 8 found in villus mesenchyme with a normal karyotype in cytotrophoblast. In the first case a normal 46,XX karyotype was found on amniocentesis, but postnatal chromosome analysis of blood from the live-born child who had malformation of the heart and kidney disclosed a low-grade mosaicism (5% trisomic cells). The second case showed trisomy 8 mosaicism on amniocentesis as well (only 3% trisomic cells), but the trisomic cell line could not be confirmed in the fetus following the abortion (karyotype 46,XX). The last case with discordance between the karyotypes of different fetal cell types was a pregnancy with non-mosaic trisomy 12 found on CVS culture, while direct preparation showed a normal karyotype (or rather an inherited, balanced reciprocal translocation). Trisomy 12 mosaicism was found on amniocentesis as well, while it could not be found in blood from cordocentesis. Low-grade trisomy 12 mosaicism was confirmed postnatally in skin fibroblasts but not in blood from the live-born

TABLE I. 192 Cases of CVS Mosaicism or Non-Mosaic Fetoplacental Discrepancy Involving a Single Autosomal Trisomy (Grouped by Distribution of the Abnormal and Normal Cell Lines)*

Type of mosaicism	Cytotrophoblast	Villus mesenchyme	Fetus[d]	Number of cases		Total (%)
CPM[a] type I	abn[c]	nor	nor	Cytotrophoblast mosaic	75	
				Cytotrophoblast non-mosaic	18	83 (43.2)
CPM type II	nor	abn	nor	Villus mesenchyme mosaic	47	
				Villus mesenchyme non-mosaic	1	48 (25.0)
CPM type III	abn	abn	nor	Cytotrophoblast mosaic; villus mesenchyme mosaic	13	
				Cytotrophoblast non-mos.; villus mesenchyme mosaic	9	
				Cytotrophoblast non-mosaic; villus mesenchyme non-mosaic	9	31 (16.1)
True fetal type IV	abn	nor	abn		—	—
True fetal type V	nor	abn	abn	Villus mesenchyme mosaic	2	
				Villus mesenchyme non-mosaic	5	7 (3.6)
True fetal type VI[b]	abn	abn	abn	Cytotrophoblast mosaic; villus mesenchyme mosaic	4	
				Cytotrophoblast non-mos; villus mesenchyme mosaic	2	
				Cytotrophoblast mosaic; villus mesenchyme non-mosaic	5	
				Cytotrophoblast non-mosaic; villus mesenchyme non-mosaic	2	13 (6.8)
Total						192 (~100.0)

*In all cases a minimum of three cell types were karyotyped.
[a]CPM, confined placental mosaicism; CPM type I, II and III refers to the definitions by Kalousek [1990].
[b]Mosaicism in at least one of the investigated cell types.
[c]Abn, mosaic or a non-mosaic abnormality.
[d]Skin biopsy, blood from fetus/neonate, fetal tissue and/or amniotic fluid cells.

TABLE II. Probabilities of Mosaic or Non-Mosaic Single Autosomal Trisomy in the Fetus Proper, According to the Combination of Cell Lineages Affected in the Placenta*

Cytotrophoblast	Villus mesenchyme	P (true)[a]
Mosaic	Normal	Type IV/(type IV + type I) = 0/(0 + 75) = 0%
Non-mosaic	Normal	do. = 0/(0 + 18) = 0%
Normal	Mosaic	Type V/(type V + type II) = 2/(2 + 47) = 4.1%
Normal	Non-mosaic	do. = 5/(5 + 1) = 83.3%
Mosaic	Mosaic	Type VI/(type VI + type III) = 4/(4 + 13) = 23.5%
Non-mosaic	Mosaic	do. = 2/(2 + 9) = 18.2%
Mosaic	Non-mosaic	do. = 5/(5 + 0) = 100%

*Calculation of probabilities are based on the figures from Table I.
[a]P(true) refers to the probability of single autosomal trisomy (mosaic or non-mosaic) in the fetus proper.

child, who had complex heart malformation and dysmorphism and mild psychomotor retardation.

DISCUSSION

Identification of those gestations with CVS mosaicism or non-mosaic fetoplacental discrepancy which are at increased risk for generalized, true fetal mosaicism would be helpful in counseling after prenatal diagnosis. Also, it would be helpful to be able to predict if some of the pregnancies with the aberration confined to the placenta, CPM, have a higher risk for uniparental disomy.

The probability of mosaic or non-mosaic trisomy in the fetus proper depended on the combination of placental lineages in which the trisomic cell line was found (Table II). Mosaic or non-mosaic trisomies found in cytotrophoblast with a normal, diploid karyotype in villus mesenchyme were never recovered in the fetus proper. However, if the trisomic cell line was found on CVS culture, either with a normal, diploid, or with a mosaic or non-mosaic trisomy in direct preparation, a risk of true fetal mosaic or non-mosaic trisomy existed. The probability of a true fetal chromosomal aberration increased if a mosaic aberration was detected on both direct preparation and long-term culture. In 5/5 gestations with a non-mosaic trisomy on CVS culture and mosaicism on direct preparation, and in 5/6 gestations with non-mosaic trisomy on CVS culture and normal

TABLE III. Distribution of Specific Single Autosomal Trisomies in Each of the Groups of Mosaicism/Discrepancy I-VI*

	CPM Number of cases[a]			True Fetal Mosaicism Number of cases[a]			
Trisomy	Type I	Type II	Type III	Type IV	Type V	Type VI	Total
2	1	9	[1]				11
3	9[b]	1					10
5	1	2					3
7	21[c] (2)	7	1 [1]				32
8	5[d] (1)	3[e]	1		1[h]		11
9	4 (1)	2 (1)				[1]	9
10		5	1				6
11	1						1
12		1			(1)[i]		2
13	7 (2)	2	2			(2)[j]	15
14	1 (1)		(1)[g]				3
15	2 (1)	1	2 (2)[g] [2]			1	11
16	1 (1)	1	(4)[g] [4]				11
17		1					1
18	8 (4)	7	4 (1)[g] [1]		1 (1)	(1)[k] [1]	29
20	5 (4)		2			1	12
21	9 (1)	3[f]			(3)	2 (4)[l]	22
22		2	(1)[g]				3
All	75 (18)	47 (1)	13 (9)[g] [9]	0	2 (5)	4 (2)[g] (5)[k] [2]	192

*The groups I-VI are identical to those listed in Table I.
[a]() = number of cases out of the total showing a non-mosaic cell line in direct or longterm. [] = number of cases out of the total showing a non-mosaic cell line in both direct and longterm.
[b]Includes one case with normal karyotype on amniocentesis and 1/100 cells with trisomy 3 in blood from the newborn.
[c]Includes one case of concomitant trisomy 7 and inherited balanced translocation.
[d]Includes one case of concomitant trisomy 8 and inherited balanced translocation.
[e]Includes one case with mosaic trisomy 8 on amniocentesis, but normal karyotype in the aborted fetus, see text.
[f]Includes one case with concomitant trisomy 21 and inherited inv(10).
[g]Non-mosaic on direct and mosaic on culture.
[h]Amniocentesis with normal karyotype, but low-grade trisomy 8 mosaicism postnatally proven in blood from the child, see text.
[i]Concomitant trisomy 12 and inherited balanced translocation; mosaic trisomy 12 in skin fibroblasts from the newborn, cordocentesis prenatally normal, see text.
[j]One case was non-mosaic on direct and mosaic on culture; the other case was mosaic on direct and non-mosaic on culture.
[k]Mosaic on direct and non-mosaic on culture.
[l]Three cases were mosaic on direct and non-mosaic on culture; one case non-mosaic on direct and mosaic on culture.

karyotype on direct, the trisomy was recovered in the fetus. In only 1/6 gestations with non-mosaic trisomy on villus culture (trisomy 9) and normal karyotype on direct, the karyotype of the fetus was normal. Nine further cases with a non-mosaic aberration in villus mesenchyme occurred, where the aberration was not confirmed in the fetus proper. In all these (trisomies 2, 7, 15, 16, and 18) the non-mosaic trisomy was also present in the cytotrophoblast. True fetal mosaicism occurred for trisomies 8, 9, 13, 18, and 21, which was not surprising, as these are all well known to be compatible with a viable fetus, and for trisomy 12 and 15, which have also previously been described in fetuses surviving beyond the first trimester [English et al., 1994; Sundberg et al., 1994]. Trisomy 20 mosaicism was in one instance recovered in the fetus post abortem, confined to costal cartilage (17% trisomic cells) with all other tissues investigated showing a normal 46,XX karyotype. Trisomy 20 mosaicism is known from amniocentesis studies and has most often been found with the trisomic cell line confined to the urinary tract or skin and not present in blood [Hsu et al., 1991]. Trisomy 22 was not confirmed as true fetal in this series, which is most likely attributable to the still relatively small numbers.

Trisomy 21 mosaicism was the one most likely to be recovered in the fetus. When encountered both in cytotrophoblast and villus culture, mosaicism for trisomy 21 was always found in the fetus as well. In only 3/12 cases where a trisomy 21 cell line was found in villus mesenchyme did the amniocenteses show a normal karyotype. All three cases were mosaic in villus mesenchyme. Non-mosaic trisomy 13, 18, and 21 in villus mesenchyme indicated fetal trisomy in 6/7 cases. The only exception was a gestation where non-mosaic trisomy 18 was found both on CVS culture and on direct preparation, while a 46,XX karyotype was found in the aborted fetus after termination (no prior amniocentesis). Of course, neither true fetal mosaicism nor maternal cell contamination can be entirely excluded as an explanation for this. Twice a trisomy 18, found on CVS culture with a normal karyotype on direct preparation, turned out to be a true fetal trisomy. These two cases would have been false negative prenatal diagnoses if CVS culture had not been analyzed. In three cases of true fetal trisomy 21 mosaicism, the trisomy was found only on CVS culture, whereas direct preparation was normal, also possible false negative prenatal diagnoses.

Confinement of the trisomy to the placenta would principally not have been detected prenatally for non-mosaic CPM type I if the CVS culture had not been analyzed (possible false positives) (for definitions of the types of CPM, see Table I). However, for 9 out of the 18 CPMs type I, the non-mosaic trisomy could be suspected to be of non-fetal origin due to the chromosome involved (7, 14, 15, 16, and 20). The non-fetal origin of the remaining non-mosaic trisomies would not necessarily have been detected (trisomies 8, 9, 13, 18, and 21). Of course, fetal mosaicism can never be entirely excluded. However, all trisomies 18, and the one trisomy 8 with non-mosaic CPM type I, had a normal karyotype on amniocentesis, and all resulted in liveborn children, phenotypically normal at birth. The CPM type I gestations with non-mosaic trisomies 9, 13, and 21 were all terminated by induced abortion without prior amniocentesis, and a normal fetal karyotype post abortem, rendering exclusion of fetal mosaicism less certain. Non-mosaic CPM type II occurred only once (trisomy 9).

As it appears from the above analysis, the individual autosomal trisomies seemed to be unevenly distributed between the different fetal and extrafetal cell lineages. The distribution of the trisomies in the CPMs in our data fits the pattern found by Wolstenholme [1995; 1996] in his reviews of case series of CPM for trisomies 2, 3, 7, 8, 9, 16, and 22 from the literature, except for trisomy 8: A consistent pattern with trisomy 2 mainly as mosaic CPM type II; trisomy 3 as mosaic CPM type I; trisomy 7 and 9 as either mosaic CPM type I or II, with a few instances of non-mosaic CPM type I and/or CPM type III, and trisomy 16 as non-mosaic CPM type III or I. Our figures for trisomy 22 CPM are too small for comparison, but for trisomy 8 our data showed mosaic CPM type I to be as likely as mosaic CPM type II, while Wolstenholme found trisomy 8 mainly as mosaic CPM type II. We found the pattern for trisomy 13 CPM similar to that of trisomy 7, and trisomy 15 similar to trisomy 16, though trisomy 15 of CPM type I and III occurred more frequently as mosaic CPM than trisomy 16. While trisomy 20 CPM was either of mosaic or non-mosaic type I, trisomy 21 CPM was most often of mosaic type I. Trisomy 18 CPM showed a very complex pattern either as mosaic type I or II, non-mosaic or mosaic type III, or non-mosaic type I. Although a complexity of mechanisms are competing in the formation of these patterns, the distribution of the aneuploid cells in CPM as diagnosed on CVS is likely to reflect the origin of the particular trisomy in the individual cases. If resulting from trisomy rescue, the CPM will most likely be of type I or III, with high proportions of trisomic cells in the cytotrophoblast, whereas mitotic errors will result in CPM type I or II with a lower percentage of trisomic cells [Wolstenholme, 1996]. According to this, meiotic errors should predominate in gestations with CPM for trisomies 15 and 16, while the trisomic cell line in CPM for trisomies 2, 3, 7, 8, and 9 should mainly originate from mitotic errors. This is in agreement with the current knowledge from published cases with CPM, tested for UPD. The risk of uniparental disomy in fetuses or children born after pregnancies with CVS mosaicism or non-mosaic fetoplacental discrepancy pertains mainly to gestations where reduction to disomy in an originally trisomic conception has taken place (risk of 1 in 3 of UPD). Despite the high frequency of CPM for trisomies 2 and 7, UPD(2)mat has only been reported twice after a prenatal diagnosis of trisomy 2 mosaicism, once with the mosaicism encountered in amniotic fluid [Harrison et al., 1995], once on CVS [Webb et al., 1996] and UPD(7)mat once after a pregnancy with CPM for trisomy 7 [Langlois et al., 1995]. Opposite to this, there have been several reports of UPD(15)mat, first by Purvis-Smith et al. [1992] and Cassidy et al. [1992] and of UPD(16)mat after pregnancies with CPM [Bennett et al., 1992; Kalousek et al., 1993; Vaughan et al., 1994]. Although such data are

biased toward pregnancies with adverse outcome, the pattern is too consistent to be explained by ascertainment bias solely.

Fetal mosaicism can result from early mitotic errors in originally diploid conceptuses, as well as from trisomy rescue. Trisomies occurring in gestations with true fetal mosaicism, such as trisomies 13, 18, and 21 showed correspondingly more puzzling patterns in the distribution of trisomic cells in the different lineages. In all fetal trisomies 13, 18, and 21 in this series, the trisomic cell line in the placenta was found either in villus mesenchyme only or in both the placental cell lineages analyzed. However, while CPM type III was relatively frequent for trisomy 18 and also occurred for trisomy 13, it never occurred for trisomy 21. The absence of trisomy 21 as CPM type III and the abundance as true fetal mosaicism type VI may reflect that a trisomy 21 cell line for reasons not known is better tolerated in the fetus than both trisomies 13 and 18, as well as trisomies 15 and 16.

In order to simplify the interpretation of our data, there are several biological problems that have not been discussed, e.g., the origin of extraembryonic mesoderm in the mesenchymal villus core (which is still a matter of debate) the presence of extrafetal cells in amniotic fluid, and the possibility of a vanishing twin. Also, a possible difference in the growth potential of the cells in culture may play a role for the phenomena observed. The three cases showing discordance between fetal karyotypes demonstrate the problems in diagnosing fetal mosaicism, and such cases fit with none of the existing classifications or models. The present paper included data on mosaic vs. non-mosaic occurrence of the trisomies rather than the exact proportion of trisomic cells. A more comprehensive analysis would need to take into account the proportion of trisomic cells as well. The proportion of abnormal cells found in CVS specimens, however, does not necessarily reflect the degree of mosaicism in the whole placenta, as chorionic villi for prenatal diagnosis is sampled only from one placental site. Definitions of the level of mosaicism may differ from one laboratory to another, and the number of cells and tissues investigated, including the information provided on single cell abnormalities, will definitely influence the number of cases and the rate of follow-up analyses. The approaches of the laboratories were not always the same; in some, long-term culture was investigated routinely in all chorionic villus samples, whereas in others it was performed only if the direct preparation was abnormal. This may result in an underestimation in the number of pregnancies with CPM type II. We reviewed our database with regard to CVS analyzed either after direct CVS preparation only or after CVS culture only, in order to see whether such a systematic error existed in the present analysis. No major differences in the distributions of the trisomies in the two preparations appeared, as compared to the distributions presented here.

In conclusion, the risk of true fetal mosaicism and the theoretical risk of uniparental disomy in the present study seemed to depend on the placental cell lineages affected and the chromosome involved. We recommend, due to the risk of fetal trisomy, amniocentesis in all gestations involving mosaic autosomal trisomy in villus mesenchyme. In gestations with mosaic or non-mosaic autosomal trisomy in both cytotrophoblast and villus mesenchyme we recommend, in order to exclude fetal trisomy and/or UPD, depending on the chromosome involved, further examination by amniocentesis, ultrasound and/or test for UPD. Our study indicated a small but not negligible risk of false negative and false positive diagnoses, if direct CVS preparation alone is used. We therefore do not recommend the sole use of direct preparation. Aside from the risk of fetal trisomy and UPD, a theoretical possibility of placental dysfunction due to extensive placental chromosomal abnormality exists. Further, chromosome-specific knowledge on the impact of CPM on the fetoplacental unit and the frequency and impact of UPD after gestations with CVS mosaicism or non-mosaic fetoplacental discrepancy is essential for the evaluation of mechanisms involved and for genetic counseling. This requires clinical follow-up of pregnancies with CPM, as well as molecular investigations for UPD. Compiling of such data is ongoing in EUCROMIC.

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APPENDIX A. CENTERS PARTICIPATING IN EUCROMIC

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